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# Isolation and Identification of Flavonoid and Related Compounds as Co-pigments from the Flowers of *Iris ensata*

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Fourteen flavonoid and related compounds which have bathochromic effect, i.e., co-pigment effect, of anthocyanin pigments were isolated from the flowers of *Iris ensata*. Of them, ten were identified as isovitexin, vitexin, isoorientin, orientin, saponarin, vicenin-2, isovitexin 2"-*O*-rhamnoside, isovitexin 2"-*O*-xyloside, mangiferin and isomangiferin, and four were partially characterized as apigenin 6,8-di-*C*-hexoside, 5,4'-dihydroxy-7,3'-dimethoxyflavone 6-*C*-glycoside and two acylated glycoses.

The flowers of cultivated *Iris ensata* Thunb. vary in color from pink or reddish purple to bluish purple, in spite of major flower anthocyanins are malvidin 3-*O*-(p-coumaroylrutinoside)-5-*O*-glucoside (ensatin) accompanied with petunidin 3-*O*-(p-coumaroyl-rutinoside)-5-*O*-glucoside (petanin) in almost cultivars (Hayashi et al. 1978, Ishikura and Yamamoto 1978, Yabuya 1991). We concluded that bluing effect of the flower color was induced by co-pigmentation with other substances which could be quantitatively detected by high performance liquid chromatography (HPLC), therefore it has been proved that some UV absorbing compounds are more abundantly present in bluish purple flowers than in reddish purple and purple ones (Yabuya et al. 1994).

In this paper, we describe the isolation and identification of co-pigment substances in the flowers of cultivars of *Iris ensata* Thunb.

## Materials and Methods

*Plant materials* *Iris ensata* Thunb. cv. "Kongo-Jo" (金剛城), which was cultivated in Kamo nursery, Kakegawa-city, Shizuoka Pref., was used as plant materials.

*Isolation of the compounds* Fresh flowers (ca. 1 kg) of *I. ensata* were extracted with methanol, filtered and evaporated to dryness *in vacuo*. After addition of water, the residue was shaken with petroleum ether and then ethyl acetate (EtOAc). EtOAc layer was evaporated to dryness, dissolved in 70% methanol and applied to a Polyamide C-200 (Wako Pure Chemicals) column (I.D. 3 × 40 cm) (solvent system: 70% MeOH). The fractions which contained compound **1** were combined and purified by Polyamide column chromatography using 50% MeOH. Compound **1** was obtained as pale yellow powder from EtOAc-MeOH. Other fractions which contained compounds **2** and **4**–**6** were applied to preparative paper chromatography

(PPC) using BAW (n-BuOH/AcOH/water = 4:1:5, upper phase), 15% AcOH and then BEW (n-BuOH/AcOH/water = 4:1:2.2) as the solvent systems. The isolated compounds were purified by Sephadex LH-20 column chromatography (I.D. 1 × 20 cm, solvent system: 70% MeOH).

On the other hand, aqueous residue was also applied to a Polyamide column (I.D. 3 × 40 cm), and the fractions were gradiently eluted with water, 20%, 40%, 60%, 80%, and finally 100% MeOH. The fractions were applied to PPC (BAW, 15% AcOH and then BEW). The isolated compounds were purified by a Sephadex LH-20 column (70% MeOH). Thus, each compound **3** and **7–13** was obtained from aqueous residue as powder or pure solutions.

*High performance liquid chromatography (HPLC)* HPLC separation of the compounds were performed with TSKgel ODS-TM column (I.D. 4.6 × 150 mm), at flow-rate: 1.0 ml/min, detection: 190–360 nm and eluent: $CH_3CN/H_2O/H_3PO_4$ (22:78:0.2) according to Hayashi et al. (1989).

*Identification of the compounds* The compounds were identified by UV spectral analysis according to Mabry et al. (1970), fast atom bombardment mass spectra (FAB-MS) using nitrobenzyl alcohol (NBA), $^1$H- and $^{13}$C-nuclear magnetic resonance ($^1$H- and $^{13}$C-NMR) spectra using dimethylsulfoxide-$d_6$ (DMSO-$d_6$), identification of their hydrolysates which were obtained by acid hydrolysis (12% aq. HCl, 100°C, 30 min.) according to Hayashi et al. (1989), and PC and HPLC comparisons with authentic specimens.

PC, UV, FAB-MS, $^1$H- and $^{13}$C-NMR and acid hydrolysis data were as follows.

Isovitexin (**1**). PC: Rf 0.72 (BAW), 0.87 (BEW), 0.46 (15% AcOH), 0.33 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark greenish yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 271, 333; +NaOMe 278, 331, 398 (inc.); +$AlCl_3$ 279, 304, 353, 381; +$AlCl_3$/HCl 280, 302, 344, 378; +NaOAc 278, 302, 390; +NaOAc/$H_3BO_3$ 272, 344. FAB-MS (NBA): $[M-H]^-$ at *m/z* 431, calcd. for $C_{21}H_{20}O_{10}$. $^1$H-NMR (270 MHz, DMSO-$d_6$): δ13.55 (1H, *s*, 5-OH), 7.93 (2H, *d*, *J* = 8.9 Hz, H-2',6'), 6.93 (2H, *d*, *J* = 8.9 Hz, H-3',5'), 6.78 (1H, *s*, H-3), 6.51 (1H, *s*, H-8), 4.85 (1H, *d*, *J* = 7.3Hz, glucosyl anomer), 3.1–4.6 (6H, *m*, glucosyl protons). Acid hydrolysis: unhydrolyzable; Wessely-Moser (W.-M.) rearrangement – occurrence of vitexin.

Vitexin (**2**). PC: Rf 0.54 (BAW), 0.71 (BEW), 0.27 (15% AcOH), 0.16 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark greenish yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 270, 333; +NaOMe 279, 330, 394 (inc.); +$AlCl_3$ 276, 304, 350, 382; +$AlCl_3$/HCl 278, 303, 343, 377sh; +NaOAc 279, 303sh, 387; +NaOAc/$H_3BO_3$ 271, 344. Acid hydrolysis: unhydrolyzable; W.-M. rearrangement – occurrence of isovitexin.

Isoorientin (**3**). PC: Rf 0.52 (BAW), 0.72 (BEW), 0.35 (15% AcOH), 0.20 (5% AcOH); UV – dark purple, UV/$NH_3$ – bright yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 257sh, 269, 350; +NaOMe 275, 409 (inc.); +$AlCl_3$ 275, 426; +$AlCl_3$/HCl 277, 296sh, 360, 385sh; +NaOAc 269, 400; +NaOAc/$H_3BO_3$ 265, 377. Acid hydrolysis: unhydrolyzable; W.-M. rearrangement – occurrence of orientin.

Mangiferin (**4**). PC: Rf 0.47 (BAW), 0.54 (BEW), 0.45 (15% AcOH), 0.33 (5% AcOH); UV – dark orange, UV/$NH_3$ – bright yellow.

Orientin (**5**). PC: Rf 0.27 (BAW), 0.28 (BEW), 0.18 (15% AcOH), 0.08 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark yellow.

Isomangiferin (**6**). PC: Rf 0.30 (BAW), 0.26 (15% AcOH), 0.15 (5% AcOH); UV – dark orange, UV/$NH_3$ – bright yellow.

Saponarin (**7**). PC: Rf 0.28 (BAW), 0.34 (BEW), 0.63 (15% AcOH), 0.51 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 272, 332; +NaOMe 273, 311, 386 (inc.); +$AlCl_3$ 279, 301, 351, 375sh; +$AlCl_3$/HCl 279, 300, 344, 375; +NaOAc 270, 391; +NaOAc/$H_3BO_3$ 271, 340. FAB-MS (NBA): $[M+H]^+$ at *m/z* 595, calcd. for $C_{27}H_{30}O_{15}$. $^1$H-NMR

(270 MHz, DMSO-$d_6$): δ13.70 (1H, *s*, 5-OH), 8.05 (2H, *d*, *J* = 8.9 Hz, H-2',6'), 7.01 (2H, *d*, *J* = 8.9 Hz, H-3',5'), 6.98 (1H, *d*, *J* = 3.6 Hz, H-8), 6.94 (1H, *s*, H-3), 5.07 (1H, *d*, *J* = 9.6 Hz, 7-*O*-glucosyl anomer), 4.77 (1H, *d*, *J* = 9.6 Hz, 6-*C*-glucosyl anomer), 3.2–4.5 (12H, *m*, diglucosyl protons). $^{13}$C-NMR (67 MHz, DMSO-$d_6$): (aglycone) δ163.5 (C-2), 102.8 (C-3), 181.9 (C-4), 156.5 (C-5), 111.0 (C-6), 163.4 (C-7), 94.3 (C-8), 161.2 (C-9), 108.1 (C-10), 121.2 (C-1'), 128.5 (C-2',6'), 116.1 (C-3',5'), 161.3 (C-4'); (6-*C*-glucosyl) δ81.1 (C-1"), 74.7 (C-2"), 70.4 (C-3"), 71.4 (C-4"), 81.6 (C-5"), 61.6 (C-6"); (7-*O*-glucosyl) δ100.5 (C-1'"), 71.6 (C-2'"), 78.4 (C-3'"), 70.2 (C-4'"), 76.3 (C-5'"), 59.8 (C-6'"). Acid hydrolysis: isovitexin and glucose; W.-M. rearrangement – occurrence of vitexin.

Vicenin-2 (**8**). PC: Rf 0.22 (BAW), 0.26 (BEW), 0.50 (15% AcOH), 0.43 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 273, 333; +NaOMe 283, 334, 400 (inc.); +$AlCl_3$ 280, 306, 352, 384sh; +$AlCl_3$/HCl 280, 304, 346, 380sh; +NaOAc 282, 304sh, 394; +NaOAc/$H_3BO_3$ 277sh, 284, 322, 345sh. Acid hydrolysis: unhydrolyzable; W.-M. rearrangement – negative.

Isovitexin 2"-*O*-rhamnoside (**9a**) and isovitexin 2"-*O*-xyloside (**9b**). PC: Rf 0.45 (BAW), 0.50 (BEW), 0.73 (15% AcOH), 0.50 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark brown. UV: $\lambda_{max}^{MeOH}$ (nm) 272, 332; +NaOMe 279, 332, 399 (inc.); +$AlCl_3$ 279, 304, 354, 377sh; +$AlCl_3$/HCl 280, 303, 346, 375sh; +NaOAc 279, 304, 391; +NaOAc/$H_3BO_3$ 272, 341. FAB-MS (NBA): $[M+H]^+$ at *m/z* 579, calcd. for $C_{27}H_{30}O_{14}$ (**9a**); $[M+H]^+$ at *m/z* 565, calcd. for $C_{26}H_{28}O_{14}$ (**9b**); $[M\text{-}O\text{-glycosyls}+H]^+$ at *m/z* 433, calcd. for $C_{21}H_{20}O_{10}$. $^1$H-NMR (270 MHz, DMSO-$d_6$); δ13.66 (1H, *s*, 5-OH), 8.05 (2H, *d*, *J* = 8.6 Hz, H-2',6'), 7.05 (2H, *d*, *J* = 8.6 Hz, H-3',5'), 6.89 (1H, *s*, H-3), 6.62 (1H, *d*, *J* = 3.6 Hz, H-8), 4.77 (1H, *d*, *J* = 9.6 Hz, glucosyl anomer), 4.38 (1H, *s*, rhamnosyl anomer), 4.15 (1H, *d*, *J* = 6.9 Hz, xylosyl anomer), 5.4–3.7 (*m*, sugar protons), 0.68 (3H, *dd*, *J* = 5.0 and 5.6 Hz, rhamnosyl Me). $^{13}$C-NMR (67 MHz, DMSO-$d_6$): (aglycone) δ163.4 (C-2), 102.8 (C-3), 181.8 (C-4), 161.2 (C-5), 108.6 (C-6), 163.5 (C-7), 93.1 (C-8), 156.4 (C-9), 102.8 (C-10), 121.2 (C-1'), 128.5 (C-2',6'), 116.1 (C-3',5'); (6-*C*-glucosyl) δ70.4 (C-1"), 81.5 (C-2"), 78.4 (C-3"), 69.4 (C-4"), 81.0 (C-5"), 65.7 (C-6"); (2"-*O*-xylosyl) δ104.2 (C-1'"), 74.2 (C-2'"), 74.7 (C-3'"), 70.6 (C-4'"), 65.8 (C-5'"); (2"-*O*-rhamnosyl) δ100.4 (C-1'"), 70.5 (C-2'"), 71.4 (C-3'"), 70.6 (C-4'"), 68.3 (C-5'"), 17.6 (C-6'"). Acid hydrolysis: isovitexin, rhamnose and xylose; W.-M. rearrangement – occurrence of vitexin.

Apigenin 6.8-di-*C*-hexoside (**10**). PC: Rf 0.22 (BAW), 0.26 (BEW), 0.45 (15% AcOH), 0.36 (5% AcOH); UV – dark purple, UV/$NH_3$ – dark yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 273, 332; +NaOMe 283, 333, 399 (inc.); +$AlCl_3$ 280, 305, 352, 383sh; +$AlCl_3$/HCl 280, 304, 345, 382sh; +NaOAc 282, 308sh, 394; +NaOAc/$H_3BO_3$ 277, 323, 345sh. FAB-MS (NBA): $[M-H]^-$ at *m/z* 593, calcd. for $C_{27}H_{30}O_{15}$. $^1$H-NMR (270 MHz, DMSO-$d_6$): δ13.78 (1H, *s*, 5-OH), 8.12 (2H, *d*, H-2',6'), 7.03 (2H, *d*, H-3',5'), 7.00 (1H, *s*, H-3), 4.89 and 4.81 (each 1H, *br*, hexosyl anomers). Acid hydrolysis: unhydrolyzable; W.-M. rearrangement – negative.

5,4'-dihydroxyl-7,3'-dimethoxyflavone 6-*C*-glycoside (**11**). PC: Rf 0.63 (BAW), 0.68 (BEW), 0.43 (15% AcOH), 0.40 (5% AcOH); UV – dark reddish purple, UV/$NH_3$ – yellow. UV: $\lambda_{max}^{MeOH}$ (nm) 277, 341; +NaOMe 282, 336, 389 (inc.); +$AlCl_3$ 279, 285sh, 305sh, 367, 380sh; +$AlCl_3$/HCl 281, 286sh, 305sh, 358, 377sh; +NaOAc 276, 335, 399; +NaOAc/$H_3BO_3$ 276, 345. Acid hydrolysis: unhydrolyzable; W.-M. rearrangement – ?.

## Results and Discussion

Fourteen UV absorbing compounds were isolated from the flowers of *Iris ensata* cv. "Kongo-jo". All of them were phenolics.

Apigenin 6-*C*-glucoside (isovitexin, **1**).

Compound **1** was obtained as pale yellow powder. UV spectral properties of **1** showed that the compound is typical flavone which possesses free 5-, 7- and 4'-hydroxyl groups. However, the compound could not be hydrolyzed by hot acid treatment, showing to be *C*-glycoside but not *O*-glycoside. FAB-MS exhibited $[M-H]^-$ at *m/z* 431, showing that 1 mol *C*-hexose is attached to apigenin nucleus. $^1$H-NMR spectra indicated the presence of six aromatic protons (H-3, -8, -2', -3', -5' and -6') and a sugar anomeric proton (δ4.85, *d*, *J* = 8.9 Hz), showing that *C*-glucosyl group is on the 6-position of apigenin. Finally, compound **1** was identified as apigenin 6-*C*-β-D-glucoside (isovitexin, Fig. 1) by PC and HPLC comparison with authentic specimen.

Of the compounds which were isolated in this experiment, isovitexin was most abundantly obtained. Isovitexin has also been detected from the flowers of *Iris setosa* Pallas and its Japanese two varieties, var. *hondoensis* Honda and var. *nasuensis* Hara (Hayashi et al. 1989) and *Iris laevigata* Fisch. (Iwashina and Ootani 1996) as a major component in Japanese *Iris* species.

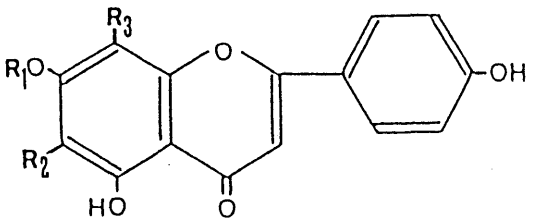
Isovitexin has been reported to be a co-pigment to cyanidin 3,5-di-*O*-glucoside (cyanin) *in vitro* (Asen et al. 1972).

Apigenin 8-*C*-glucoside (vitexin, **2**).

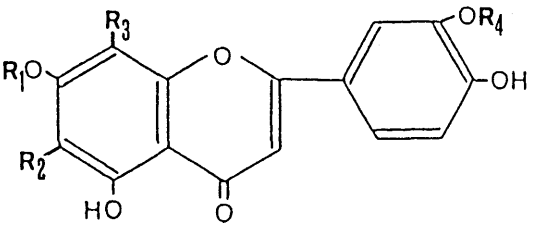
Compound **2** was also *C*-glycosylflavone possessing free 5-, 7- and 4'-hydroxyl groups. The flavonoid could not be hydrolyzed, but isovitexin, which was characterized by PC and HPLC comparison with authentic specimen, was produced by hot acid treatment (Wessely-Moser rearrangement, Markham 1982). Original glycoside was identified as apigenin 8-*C*-glucoside (vitexin, Fig. 1) by direct PC and HPLC comparison with authentic specimen.

Vitexin has been found in the flowers of *I. setosa* (Hayashi et al. 1989), *Iris rossii* Baker (Hayashi et al. 1980) and *I. laevigata* (Iwashina and Ootani 1996).

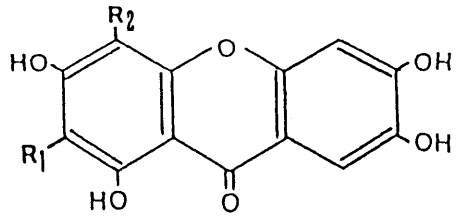
Vitexin has been reported to be co-pigment to delphinidin 3-*O*-(p-coumaroylrutinoside)-5-*O*-glucoside (delphanin) in "Prof. Blaauw Iris", a hybrid of *Iris tingitana* Boiss. & Reut. (Asen et al. 1970), but the flavonoid was detected as a minor component in the flower of *I. ensata*.




$R_1 = R_3 = H$, $R_2$ = glucosyl: isovitexin (**1**)
$R_1 = R_2 = H$, $R_3$ = glucosyl: vitexin (**2**)
$R_1 = R_2$ = glucosyl, $R_3 = H$: saponarin (**7**)
$R_1 = H$, $R_2 = R_3$ = glucosyl: vicenin-2 (**8**)
$R_1 = R_3 = H$, $R_2$ = rhamno(1→2)glucosyl: isovitexin 2"-*O*-rhamnoside (**9a**)
$R_1 = R_3 = H$. $R_2$ = xylo(1→2)glucosyl: isovitexin 2"-*O*-xyloside (**9b**)
$R_1 = H$, $R_2 = R_3$ = hexosyl: apigenin 6,8-di-*C*-hexoside (**10**)




$R_1 = R_3 = R_4 = H$, $R_2$ = glucosyl: isoorientin (**3**)
$R_1 = R_2 = R_4 = H$, $R_3$ = glucosyl: orientin (**5**)
$R_1 = R_4$ = Me, $R_2$ = glycosyl, $R_3 = H$: 5,4'-dihydroxy-7,3'-dimethoxyflavone 6-*C*-glycoside (**11**)




$R_1$ = glucosyl, $R_2 = H$; mangiferin (**4**)
$R_1 = H$, $R_2$ = glucosyl: isomangiferin (**6**)

Fig. 1. Chemical structures of flavonoid and related compounds isolated from the flowers of *Iris ensata*.

Luteolin 6-*C*-glucoside (isoorientin, **3**) and luteolin 8-*C*-glucoside (orientin, **5**).

It was shown by UV spectral analysis and hot acid treatment that compound **3** was a *C*-glycosylflavone possessing free 5-, 7-, 3'-, and 4'-hydroxyl groups. Finally, flavonoid **3** was identified as luteolin 6-*C*-glucoside (isoorientin, Fig. 1) by PC and HPLC comparison with authentic specimen.

Component **5** which was present in a very small amount was also confirmed to be luteolin 8-*C*-glucoside by PC and HPLC comparison with authentic orientin (Fig. 1).

Isoorientin has also been known as a co-pigment substance together with vitexin, swertisin, sertiajaponin and *O*-xylosylswertisin in Prof. Blaauw Iris (Asen et al. 1970).

1,3,6,7-tetrahydroxyxanthone 2-*C*-glucoside (mangiferin, **4**) and 1,3,6,7-tetrahydroxyxanthone 4-*C*-glucoside (isomangiferin, **6**).

From the color reaction (dark orange under UV light and bright yellow under UV after exposure to fuming ammonia), these compounds which were present in an extremely small quantity were characterized as xanthones (Hayashi et al. 1989). They were identified as 1,3,6,7-tetrahydroxyxanthone 2-*C*-glucoside (mangiferin, Fig. 1) and its isomer, 1,3,6,7-tetrahydroxyxanthone 4-*C*-glucoside (isomangiferin, Fig. 1) by direct comparisons with authentic specimens using PC and HPLC.

Mangiferin has been reported to be major component of *Iris pseudacorus* L. (Bate-Smith and Harborne 1963) and *I. setosa* (Hayashi et al. 1989), and has been known as one of the major co-pigments in some *Iris* flowers (Bate-Smith and Harborne 1963).

Isovitexin 7-*O*-glucoside (saponarin, **7**).

Component **7** was obtained as greyish yellow powder. UV spectral properties of **7** were those of *C*-glycosylflavone possessing free 5-, 4'-hydroxyl and a substituted 7-hydroxyl groups. Isovitexin and glucose were liberated by acid hydrolysis. $^{1}$H-NMR spectra exhibited six aromatic protons and two glucosyl anomeric protons (δ5.07, *d*, *J* = 9.6 Hz, 7-*O*-glucosyl) and (δ4.77, *d*, J = 9.6 Hz, 6-*C*-glucosyl), showing β-linkage of both *C*- and *O*-glucosyls. FAB-MS indicated $[M+H]^+$ at *m/z* 595, showing the attachment of 1 mol glucose to isovitexin. From the results described above, compound **7** was regarded to be isovitexin 7-*O*-β-D-glucoside (saponarin, Fig. 1). $^{13}$C-NMR data of **7** also agreed with that of literature (Österdahl 1978, Agrawal and Bansal 1989).

Saponarin has been presumed to be a co-pigment to malvidin 3,5-di-*O*-glucoside (malvin) in the flowers of *Strongylodon macrobotrys* A. Gray (Leguminosae) (Iwashina et al. 1984).

Apigenin 6,8-di-*C*-glucoside (vicenin-2, **8**) and apigenin 6,8-di-*C*-hexoside (**10**).

Two compounds **8** and **10** were also *C*-glycosylflavones which attach the same kind of *C*-glycosyl groups to both 6- and 8-positions, since these flavonoids were neither hydrolyzed nor occurred the isomer by hot acid treatment. By UV spectral analysis, they showed the presence of free 5-, 7- and 4'-hydroxyl groups. PC and HPLC data of **8** completely agreed with those of authentic vicenin-2, i.e., apigenin 6,8-di-*C*-glucoside (Fig. 1).

On the other hand, FAB-MS and $^{1}$H-NMR of component **10** which was obtained as pale yellow powder exhibited $[M-H]^-$ at *m/z* 593, and the presence of H-3, -2', -3', -5' and -6' protons and the absence of H-6 and -8 protons, showing the attachment of each 1 mol *C*-hexose to 6- and 8-positions of apigenin.

Component **10** was hence regarded to be apigenin 6,8-di-*C*-hexoside, probably apigenin 6,8-di-*C*-galactoside.

Isovitexin 2"-*O*-rhamnoside (**9a**) and isovitexin 2"-*O*-xyloside (**9b**).

Component **9** appeared as a single spot on the paper chromatogram, but was divided into two peaks (**9a** and **9b**) which gave slightly different retention times, by HPLC analysis. UV spectra of the mixture

showed the presence of free 5-, 7- and 4'-hydroxyl groups. Isovitexin, rhamnose and xylose were liberated by acid hydrolysis. FAB-MS of component **9** exhibited $[M+H]^+$ at *m/z* 579 and 565, showing the attachment of each 1 mol rhamnose (**9a**) and xylose (**9b**) to 6-*C*-glucosyl group of isovitexin, respectively. $^1$H-NMR spectra showed the presence of *C*-β-D-glucosyl (δ4.77, *d*, *J* = 9.6 Hz), *O*-α-L-rhamnosyl (δ4.38, *s*) and *O*-β-D-xylosyl (δ4.15, *d*, *J* = 6.9 Hz) anomeric protons (Markham and Geiger 1994) in addition to six aromatic protons and a rhamnosyl Me proton. It was shown that each rhamnose and xylose was on 2"-OH of 6-*C*-glucosyl group, by comparisons of $^{13}$C-NMR data among the mixture and authentic isovitexin 2"-*O*-xyloside (Matsuzaki et al. 1990), vitexin 2"-*O*-rhamnoside (Nikolov et al. 1982), isoorientin 6"-*O*-glucoside (Chulia and Mariotte 1985) and isoorientin 6"-*O*-arabinoside (Hostettman and Jacot-Guillarmod 1976). From the results described above, component **9** was proved to be the mixture of isovitexin 2"-*O*-α-L-rhamnoside (**9a**) and isovitexin 2"-*O*-β-D-xyloside (**9b**) (Fig. 1).

5,4'-dihydroxy-7,3'-dimethoxyflavone 6-*C*-glycoside (**11**).

UV spectra indicated that a minor component **11** was *C*-glycosylflavone possessing free 5- and 4'-hydroxyl and substituted 7- and 3'-hydroxyl groups. The compound could not be hydrolyzed by hot acid treatment. It was presumed by PC behaviors that *C*-glycosyl group is on the 6-position of luteolin. Thus, compound **11** was partially characterized as 5,4'-dihydroxy-7,3'-dimethoxyflavone 6-*C*-glycoside (Fig. 1).

Besides the compounds described above, two acylated glycoses (**12** and **13**) were obtained as powder from the flowers of *I. ensata*. They liberated unknown one (**12**) or two (**13**) aromatic acids in addition to glucose and rhamnose by acid hydrolysis. The determination of their chemical structures were now in progress.

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岩科　司，亀之園浩治，藪谷　勤: **ハナショウブの花のコピグメントとしてのフラボノイドとその関連物質の分離と同定**

ほとんどのハナショウブ品種の花色はその主要アントシアニンが ensatin （malvidin 3-*O*-(p-coumaroylrutinoside) 5-*O*-glucoside）であるにもかかわらず，赤紫～紫青色までの変化がある．これらの花色を定量的に HPLC で分析すると，紫青色の品種群に複数の紫外線吸収物質が多量に存在する．これらが花色を青色へと変化させる効果，すなわちコピグメント効果のある物質群であることはすでに私たちによって報告されている．

本研究はこれらのコピグメント様物質の分離と同定を目的に行った．

ハナショウブ品種“金剛城”の花のメタノール抽出物からは14種類の紫外線吸収物質が分離された．これらはいずれもフラボノイドとキサントンの *C*-配糖体で，UV 吸収スペクトル，FAB-MS，$^{1}$H- および $^{13}$C-NMR スペクトル，酸加水分解とその生成物の同定，および基準標品とのペーパークロマトおよび HPLC での比較によって isovitexin, vitexin, isoorientin, orientin, saponarin, vicenin-2, isovitexin 2″-*O*-rhamnoside, isovitexin 2″-*O*-xyloside（以上フラボノイド），mangiferin および isomangiferin（以上キサントン）と同定された．残りの 4 種類は apigenin 6, 8-di-*C*-hexoside, 5,4′-dyhydroxy-7, 3′-dimethoxyflavone 6-*C*-glycoside, および 2 種類のアシル化糖と定性された．

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